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Applicant: Blakesley et al. Group Art Unit: 1637
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For: ARCHIVING OF VECTORS
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL DECLARATION OF DR. WALTER KING PURSUANT TO

37 CFR 1.132

I, Dr. Walter King, declare as follows:

1. Further to my Declaration of March 19, 2009 (mailed March 23, 2009), I am presently the Vice President of Research & Development at GE Healthcare in Cardiff, Wales, United Kingdom. I have been employed in this position since April 25, 2009. Previously (2006-2009), I was the Vice President of Research & Development at Whatman plc (which became part of the Life Sciences Division of GE Healthcare USA during this time), and I was based in the facility in Sanford, Maine. I am an experienced Ph.D. with an extensive background in Bacteriology & Immunology, Virology, and Microbiology & Urology. I am responsible for managing all aspects of global research and development activities at GE Healthcare, including development and implementation of the product portfolio. Prior to my positions with Whatman plc (now part of the Life Sciences Division of GE Healthcare USA), I had previously (2005-2006) been employed as a Director of the Applications Development Group at Nanosphere, Inc., in Northbrook, IL, where I was in charge of development of In

Vitro Diagnostic (IVD) products for genetic and infectious disease testing using single nucleotide polymorphism detection technology in patient samples without amplification. Prior to that, I had been a Senior Director of Product Development in the Vysis Diagnostic Division and Molecular Oncology Program Director at Abbott Laboratories (2002-2005) in Downers Grove, IL, where I was in charge of development of microarray IVD products for molecular cytogenetics and oncology; and a Director of the Genomic Microarray Platform program (1999-2001; genomic array-based assay products for the detection of amplification and deletions for solid tumors, leukemias, lymphomas and prenatal applications) and a Senior Manager of Assay Development (1995-1998; IVD's for Her-2 detection in breast cancer [PMA approval], multiplex aneuploidy detection in bladder cancer [510K approval], and pre-implantation testing in blastomeres and polar bodies) at Vysis, Inc. in Downers Grove, IL. I was also a Senior Scientist in Assay Development (1986-1995; assay formats and chemistries for an automated clinical analyzer; amplification chemistry for detection of respiratory, gastrointestinal and sexually transmitted disease panels; manual non-isotopic probe-based test for detection of *Listeria*, *Salmonella* and *E. coli*) at Gene-Trak Systems, Inc., in Framingham, MA; and was employed in the Biotechnology Group (1985-1986; development of nucleic acid probe technology for the detection of clinical pathogens) of AMOCO Corporation in Naperville, IL. I have been active in the development of various products, have been listed as an inventor on several patents, and have co-authored over thirty publications. I hold a Bachelor of Arts (1973) from the Department of Bacteriology and Immunology of the University of California in Berkeley, CA. I hold a Ph.D. in Virology (1980) from the University of Chicago in Chicago, IL. I served as a Postdoctoral Fellow in the Department of Medicine at the University of Chicago (1980-1982), where I identified the transforming region in the Epstein-Barr Virus (EBV) genome, and in the Departments of Microbiology and Urology at Columbia University Medical Center in New York, NY (1982-1985), where I identified genes involved in the differentiation of embryonal carcinoma cells. I am a member of the American Association for Cancer Research.

2. The subject application discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) providing a dry

matrix or solid medium, wherein said dry matrix or solid medium further comprises: i) a weak base; ii) a chelating agent; and iii) an anionic surfactant or an anionic detergent; b) contacting the matrix or solid medium with a sample comprising a host cell containing said plasmid or plasmids; and c) isolating all or a portion of said plasmid or plasmids from said matrix or solid medium. It also discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) contacting a solid medium with a sample comprising a host cell containing said plasmid, wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; ii) a weak base; iii) a chelating agent; and iv) an anionic surfactant or an anionic detergent; b) releasing the plasmid from the host cell and onto said medium; and c) isolating said plasmid from said medium. It also discloses among other things and claims a method of isolating or purifying one or more plasmids from a host cell comprising: a) contacting a solid medium with a sample comprising a host cell containing said plasmid, wherein said solid medium protects against degradation of said plasmid and wherein the solid medium comprises: i) a polymeric matrix comprising a cellulose-based matrix, a micromesh synthetic polymer matrix, or a micromesh synthetic plastic matrix; and ii) a composition sorbed to the polymeric matrix, wherein the composition comprises: a weak base; a chelating agent; and an anionic surfactant or an anionic detergent; b) lysing the host cell; c) releasing the plasmid from the host cell and onto said medium; and d) isolating said plasmid from said medium.

3. As noted previously, for many projects, generation of numerous DNA samples from biological specimens is routine. Traditionally, however, handling and archiving a large collection can become a logistical problem for the laboratory. Previously, there had often been a need to use organic solvents or harsh chemicals or to store samples in a freezer. One solution, used in forensic labs, was the bloodstorage medium FTA[®] Cards. An FTA[®] Card could be used to store genomic DNA in the form of dried spots of human whole blood, the cells of which were lysed on the paper. Before analysis of the captured genomic DNA, a few washing steps would be used to remove the stabilizing chemicals and cellular inhibitors of

enzymatic reactions, but the genomic DNA would largely remain on the card. Isolated bacterial DNAs spotted on FTA[®] Cards could be an efficient system for storage and retrieval as well, followed by downstream applications, such as PCR ribotyping. Further, purified plasmid DNA was spotted on treated paper and recovered. Nevertheless, there was a need for a simplified method of isolation and storage of plasmid DNA directly from cells onto a dry matrix or solid medium without prior isolation of the plasmid DNA from the host, followed by its subsequent isolation from the matrix or solid medium.

4. The current invention addresses these concerns and many other issues as well, such as protection from degradation.

5. I have reviewed the Supplemental Amendment, mailed July 7, 2009 ("Supplemental Amendment"), which was discussed during the Telephone Interview of August 5, 2009 ("Telephone Interview").

6. As described in the Supplemental Amendment (see p. 9) and as discussed during the Telephone Interview, Rogers & Burgoyne describes experiments with genomic DNA. Although Kahn mentions how plasmid DNA can be separated from genomic DNA on the basis of its smaller size or its unique properties of it being a covalently closed circular DNA or, in other words, less complex than genomic DNA, this reference actually teaches away from the present invention. For example, as previously noted, plasmid DNA behaves differently from genomic DNA based on its composition and its structure. It would not be expected that less complex DNA would interact with a solid matrix in the same manner as genomic DNA, so it would not be intuitive that plasmid DNA could be isolated on a solid matrix. In point of fact, prior separation of plasmid DNA from genomic DNA is preferred, as shown in Old & Primrose and other references previously submitted. Burgoyne, however, only demonstrates DNA isolation directly from cells for genomic DNA, whereas the isolation of plasmid DNA requires pre-purification prior to contact with the solid medium. It would not have been at all clear, much less a matter of reasonable expectation, that isolation of

plasmid DNA directly from cells (despite the presence of genomic DNA) would be possible, particularly given the low copy numbers of some plasmids (see, e.g., Lerner & Inouye, "Low copy number plasmids regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability," Nucl. Acids Res. 18(15): 4631 (1990), which was previously submitted). One of ordinary skill in the art would have recognized that plasmid DNA in general constitutes a small fraction of DNA in the host cell and that the situation would be exacerbated where the plasmid copy number is low. In such a situation, efficient retention and recovery would be important, while those of ordinary skill in the art would have considered removal of RNA and genomic DNA to have been a usual step during isolation procedures (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed.), Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY: 1989), pp. 1.1-1.11, 1.21-1.24, and 1.51).

7. In the context of this application, "Archiving of Vectors," plasmid vectors are small, circular molecules of double-stranded DNA derived from natural plasmids that occur in bacterial cells. The number of copies of a particular plasmid in a cell varies from one plasmid to the next depending on the mechanism by which replication is regulated. Low copy-number plasmids are present in 1-2 copies per cell. The bacterial genome of *E. coli*, which is the most common host for plasmid vectors containing recombinant DNA segments, is approximately 4.6 million base pairs, while their corresponding plasmids, such as the ColE1 family, are about 3 orders of magnitude smaller.

8. This discrepancy in mass poses a significant technical challenge in the development of a suitable matrix for the efficient recovery of plasmid DNA where the binding capacity per unit area is limited. Because one of skill in the art was dealing with a poorly understood mechanism of macromolecular binding in a dried spot, the binding capacity of such treated matrices in complex media, like bacterial cultures, is not limited to just DNA, but also proteins and other macromolecules present in the culture, which further limits the number of "available sites" on the matrix.

9. Burgoyne demonstrated recovery of previously purified plasmid from FTA[®] medium, i.e. where all the input DNA was uniformly plasmid DNA and devoid of other macromolecules present in the original bacterial culture. The following is an example how this mass discrepancy becomes problematic for the recovery of non-purified plasmids in a bacterial culture:

If 0.1 microgram of purified pUC19 plasmid DNA (the plasmid example used by Burgoyne) was applied to FTA[®] medium, this would correspond to approximately 3.4×10^{10} molecules on the paper. If 99% (Burgoyne termed "approximately 100%") of the DNA were detected, then approximately 1% or 3.4×10^8 molecules of plasmid DNA might not be detected. Conversely 0.1 microgram of *E. coli* DNA from a culture containing a low copy plasmid only corresponds to approximately 2×10^7 molecules of genomic *E. coli* DNA and the same number of plasmid DNA molecules, which is more than an order of magnitude lower in target molecules than the purified plasmid scenario. In the context of finite binding capacity of any substrate where the mass of DNA is independent of genome size, the 1.9×10^7 molecules of plasmid DNA which co-purified with the genomic DNA would be below the total number of plasmid DNA inputted on the paper.

10. In situations where the mass of the plasmid is several orders of magnitude lower than that of the host DNA, the efficient recovery and detection is not at all feasible from a mass perspective.

11. Therefore, the teachings of Rogers, Burgoyne, and Kahn, either alone or in combination, would not have suggested the present invention to one of ordinary skill in the art with any reasonable expectation of success.

12. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 14 September 2009

Walter King
Walter King, Ph.D.